EXTRACELLULAR AND STANDARD UNIVERSITY OF WISCONSIN SOLUTIONS PROVIDE EQUIVALENT PRESERVATION OF MYOCARDIAL FUNCTION

The deleterious effect of hyperkalemic cardioplegic solutions on coronary endothelium has been documented and has also been demonstrated with University of Wisconsin solution. We evaluated a new extracellular University of Wisconsin formulation for efficacy in heart preservation. Six neonatal piglet hearts were arrested with and stored in the standard intracellular University of Wisconsin solution (group 1: K^+ 125 mEq/L, Na^+ 29 mEq/L). Six piglet hearts were preserved for 24 hours with an extracellular University of Wisconsin solution that differed only in the concentrations of potassium and sodium (group 2: K^+ 25 mEq/L, Na^+ 129 mEq/L). Hearts underwent modified reperfusion with leukocyte-depleted aspartate-glutamate enriched blood cardioplegic solution followed by conversion to a left-sided working mode on a Langendorff circuit with perfusion from a support pig. Stroke work index was calculated at left ventricular end-diastolic pressures of 3, 6, 9, and 12 mm Hg. Sixty minutes after reperfusion, there was no significant difference in stroke work index between group 1 (16.4 ± 1.9 × 1000 erg/gm) and group 2 (15.3 ± 2.7 × 1000 erg/gm). There was also no significant difference in high-energy phosphate stores or myocardial water content between the two groups. Extracellular University of Wisconsin solution provides myocardial preservation equivalent to standard University of Wisconsin solution while preventing exposure of coronary endothelium to high levels of potassium, which justifies its use in clinical heart transplantation. (J THORAC CARDIOVASC SURG 1995; 110:738-45)

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The extension of acceptable ischemic time in heart transplantation with improved myocardial preservation techniques has expanded the donor pool and may facilitate the future use of HLA matching.[1]

The University of Wisconsin (UW) solution has become recognized as the "gold standard" for kidney and liver preservation, providing superior results in both laboratory and clinical studies.[2-4] The UW solution contains macromolecular impermeant molecules to prevent cell swelling, a buffer to prevent intracellular acidosis, a colloid to facilitate vascular flushout, and several additional components theoretically important in organ preservation. In addition, the solution has a high potassium concentration (125 mEq/L) to approximate the intracellular environment and theoretically prevent Na-K adenosinetriphosphatase (ATPase) activity and its resultant cellular accumulation of water and depletion of adenosine triphosphate (ATP) stores.[5]

Encouraging laboratory results that showed complete functional recovery in hearts preserved with UW solution for as long as 24 hours[6-8] prompted interest in the use of UW for clinical heart preservation. In our laboratory, hearts preserved with UW for 12 hours demonstrated functional recovery of 100% of nonischemic control hearts, compared with 33% for hearts preserved with Stanford cardioplegic solution.[9] A randomized clinical trial that compared UW and Stanford solution for heart preservation at

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our institution resulted in our adoption of UW for heart preservation in late 1990.[10]

Concern has been expressed about the high potassium concentration of UW solution with respect to its possible deleterious effects on coronary endothelium. The damaging effects of hyperkalemic cardioplegic solutions are documented[11-13] and these effects have recently been associated with UW solution.[14] In our laboratory, we have demonstrated that multidose UW solution produces a near-complete loss of endothelium-dependent vasodilatation and nitric oxide release.[15]

Implications of endothelial dysfunction include impaired autoregulation of myocardial perfusion[16] and increased propensity toward thrombosis[17] and atherosclerosis.[18, 19] We recently conducted a retrospective study that compared the prevalence of cardiac allograft vasculopathy (CAV) in the final 100 recipients in our program to have the donor hearts preserved with Stanford cardioplegia (potassium 30 mEq/L) with the prevalence in the first 100 recipients to have the donor hearts preserved with UW solution. The prevalence of cardiac allograft vasculopathy at 24 months of follow-up was 21% in the UW group compared with 10.5% in the Stanford group.[20] Preservation solution was the only significant ($p = 0.001$) factor influencing the prevalence of cardiac allograft vasculopathy.

On the basis of this finding, we sought a heart preservation solution that would provide the same excellent myocardial preservation as standard UW solution and provide improved endothelial preservation. We hypothesized that reducing the potassium concentration to 25 mEq/L and raising the sodium concentration by an equal amount would not induce the endothelial dysfunction caused by standard UW solution and would simultaneously maintain excellent preservation of myocardial function.

Methods

Experimental design. Two groups were evaluated. The first group ($n = 6$) served as a control group and included piglet hearts that were arrested with and stored in standard high-potassium (125 mEq/L) UW solution. The experimental group ($n = 6$) was used to evaluate the use of a low-potassium (25 mEq/L) UW formulation for arrest and storage. The final composition of each solution is listed in Table I. To ensure uniformity of the other components between groups, a single stock solution was made in our laboratory to which potassium and sodium were added to produce the final formulations. All personnel performing the arrest and reperfusion procedures were blinded as to which UW formulation was being used for arrest and storage.

Table I. *Composition of the preservation solutions*

Component	Solution	
	Extracellular UW (low K)	*Intracellular UW (high K)*
Pentafraction (%)	5	5
Lactobionate (mmol/L)	100	100
Raffinose (mmol/L)	30	30
Adenosine (mmol/L)	5	5
$MgSO_4$ (mmol/L)	5	5
Glutathione (mmol/L)	3	3
Allopurinol (mmol/L)	1	1
KH_2PO_4 (mmol/L)	25	25
NaOH (mmol/L)	129	29
KOH (mmol/L)	—	104
Osmolarity (mmol/L)	300-320	300
pH	7.6-7.8*	7.6-7.8

Pentafraction was a generous gift from DuPont Pharmaceuticals, Wilmington, Del.

*pH of extracellular (low-potassium) solution adjusted with the addition of hydrochloric acid.

Arrest. Our methods of neonatal piglet heart arrest, storage, and reperfusion have previously been described and were used in this study.[8, 9] All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). Neonatal Duroc piglets (24 to 72 hours old) were anesthetized and the lungs mechanically ventilated with an inspired oxygen fraction of 1.0 at a rate of 15 to 20 cycles/min. The heart and great vessels were exposed, and the hemiazygous vein and left subclavian artery were ligated. After heparin (1000 units) and cefazolin sodium (100 mg) were injected into the main pulmonary artery, the innominate artery was cannulated and connected to a pressure monitor. Arrest was initiated with ligation of the superior and inferior vena cavae, crossclamping of the descending thoracic aorta, and infusion of high- or low-potassium UW solution at 4° C into the aortic root via the innominate artery cannula. Simultaneously with the infusion of UW, the left pulmonary veins and the main pulmonary artery were incised to decompress the heart. The cardioplegic solution was infused for 2 minutes at a pressure of 40 to 50 mm Hg with concurrent topical cooling.

Storage. Cardiectomy was done by division of the vena cavae, pulmonary veins, aorta, and pulmonary artery. Immediately after excision the hearts were immersed in the same solution used for arrest at a temperature of 4° C. The innominate artery cannula was left in place during storage. Each container was sealed and packed in ice, and a temperature of 0.5° to 1° C was maintained for 24 hours.

Reperfusion. Reperfusion was achieved with use of an isolated blood-perfused working left-sided heart model,

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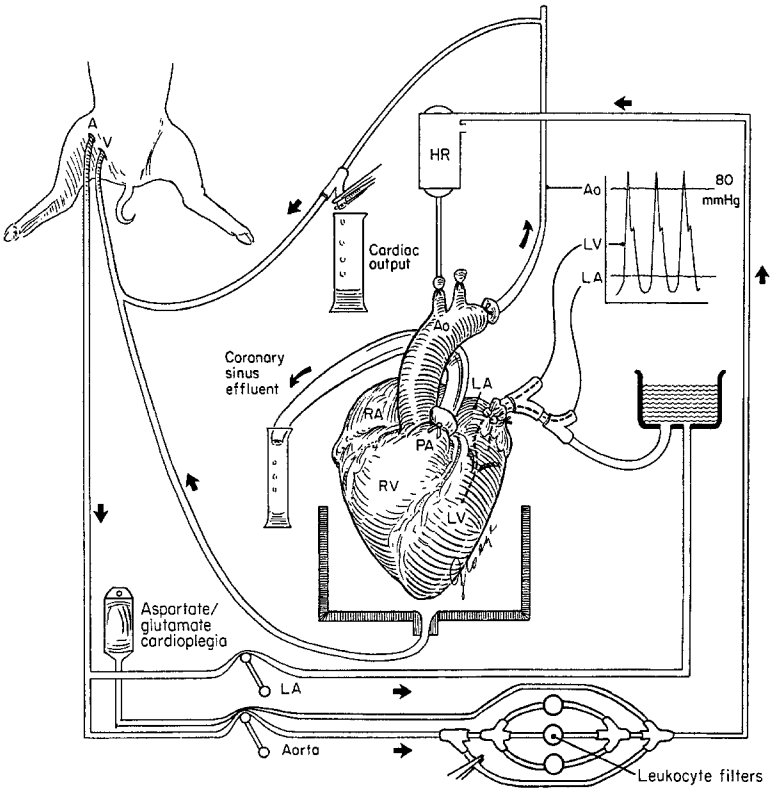

Fig. 1. Diagram of isolated perfusion circuit. Blood from support animal can be pumped to either aortic root or to adjustable-height reservoir that supplies left atrium. Afterload is determined by adjustable-height aortic column. Cardiac output can be measured by collecting coronary sinus effluent via pulmonary artery cannula plus overflow from aortic column. *A,* Femoral artery; *V,* femoral vein; *LA,* left atrium; *LV,* left ventricle; *RA,* right atrium; *RV,* right ventricle; *PA,* pulmonary artery; *Ao,* aorta; *HR,* heated reservoir containing blood cardioplegia (see text).

which is illustrated in Fig. 1. This model allows perfusion both via the innominate artery in the nonworking mode and via the left atrium when converted to the working mode.

Adolescent (4 to 6 months old, 60 to 70 kg) Duroc support pigs used for reperfusion were anesthetized and the lungs ventilated with 100% oxygen. Arterial blood gas values were obtained every 20 minutes, and appropriate ventilator adjustments or correction of metabolic acidosis with sodium bicarbonate, or both, were done. After cannulation of the right femoral artery and vein, anticoagulation was achieved by infusion of 40,000 units heparin and was maintained with the addition of 10,000 units/hr.

Blood from the support animal was directed through a roller pump to a heated reservoir and bubble trap (model BCD Advanced, Shiley Inc., Irvine, Calif.) and to a heated height-adjustable reservoir. Reperfusion and suspension of the heart was achieved by connection of the innominate artery cannula to the heated reservoir and bubble trap while distal aortic crossclamping was maintained. For the first 10 minutes the reperfusate was modified by the addition of aspartate-glutamate enriched cardioplegic solution and by leukocyte depletion. Whole blood and aspartate-glutamate enriched cardioplegic solution were delivered to the roller pump in a 4:1 ratio and mixed immediately after the pump. Leukocyte depletion was accomplished by directing the enriched blood through two filters (model RC100, Pall Filter Corp., Glen Cove, N.Y.) placed in parallel between the roller pump and the heated reservoir and bubble trap. The blood flow was diverted to a new pair of leukocyte filters after 5 minutes of reperfusion to ensure complete depletion. After 10 minutes of reperfusion, aspartate-glutamate cardioplegic solution delivery and leukocyte depletion were terminated. Blood samples were drawn from the support pig arterial cannula just before reperfusion and from the innominate artery cannula after 2 and 8 minutes of reperfusion to determine the degree of leukocyte depletion.

Modified reperfusion was begun via the innominate artery cannula at 50 mm Hg and 37° C for 10 minutes, followed by 10 minutes of whole blood reperfusion at 80 mm Hg before conversion to the working mode. The aorta was cannulated with a 16-gauge perfusion cannula after modified reperfusion was terminated and connected to an open adjustable column. Blood from the pulmonary artery was collected to reflect coronary sinus effluent. Blood from the aortic column and pulmonary artery was filtered and returned to the support animal.

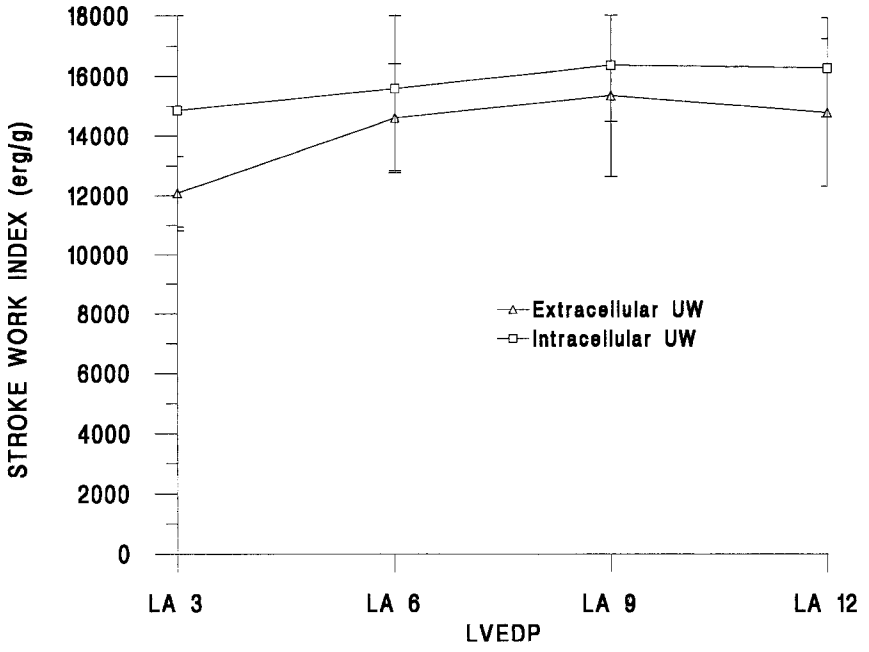

Fig. 2. No significant difference in SWI was noted between extracellular UW and intracellular UW solutions at each level of LVEDP. *LA,* Left atrial pressure (mm Hg).

To convert hearts to working mode perfusion, the left atrium was opened between the right and left pulmonary veins, and the septum was inspected for the presence of a patent foramen ovale. If present it was closed with the use of 5-0 polypropylene suture. A purse-string pledget-supported 4-0 polypropylene suture was placed around the left atriotomy, which was then cannulated and connected to a height-adjustable 37° C water-jacketed reservoir. The reservoir was supplied with arterial blood from a second roller pump.

Fiberoptic transducer-tipped catheters (1.3 mm outer diameter, model 100-4, Camino Laboratories, San Diego, Calif.) were advanced into the left atrium and the left ventricle for pressure monitoring and recording.

Evaluation of functional recovery. After 20 minutes of innominate artery perfusion and 30 minutes of working-mode perfusion, left ventricular functional assessment was made. Function curves were determined by measurement of cardiac output (sum of aortic column overflow and right ventricular return) at a constant aortic pressure of 80 mm Hg and at left ventricular end-diastolic pressures (LVEDPs) of 3, 6, 9, and 12 mm Hg. The stroke work index (SWI; ergs per gram) was calculated from the following equation:

$$SWI = \frac{(MAP - LVEDP) \times CO}{HR \times WT} \times 1333$$

where MAP is mean aortic pressure (80 mm Hg), CO is cardiac output (milliliters per minute), HR is heart rate (beats per minute), and WT is weight (grams). Functional assessment was done at 30, 60, 90, and 120 minutes after initiation of working-mode perfusion.

Myocardial high-energy phosphate assessment. After functional determinations were completed, transmural biopsy samples of the left ventricular free wall were obtained and rapidly frozen in liquid nitrogen. Measurements for ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), and creatinine phosphate were made with the use of high-pressure liquid chromatography.

Ultrastructural assessment. Transmural left ventricular biopsy specimens were taken at the completion of the functional assessment and fixed in 2.5% glutaraldehyde. One-micron sections were stained with uranyl acetate and lead citrate and examined on a Zeiss EM 109 microscope (Zeiss, Munich, Germany). Ultrastructural changes were evaluated in a blinded fashion.

Myocardial water content. Left ventricular biopsy specimens were weighed before and after desiccation at 100° C for 24 hours. The myocardial water content was calculated from the wet and dry weights.

Results

Functional recovery. Function curves are diagrammed in Fig. 2. Comparisons of SWI between groups were made at an LVEDP of 9 mm Hg, which corresponded to the peak of the Starling curve. Comparison between groups was made with functional data obtained 60 minutes after initiation of working-mode perfusion. The mean function of the hearts that received low-potassium UW (SWI 15.3 ± 2.7 × 10E3 erg/gm) was not different from

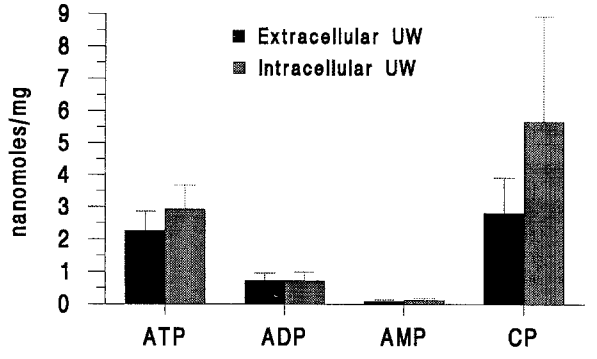

Fig. 3. No significant difference between extracellular UW and intracellular UW solutions was noted for high-energy phosphates ATP, ADP, AMP, and creatine phosphate *(CP)*.

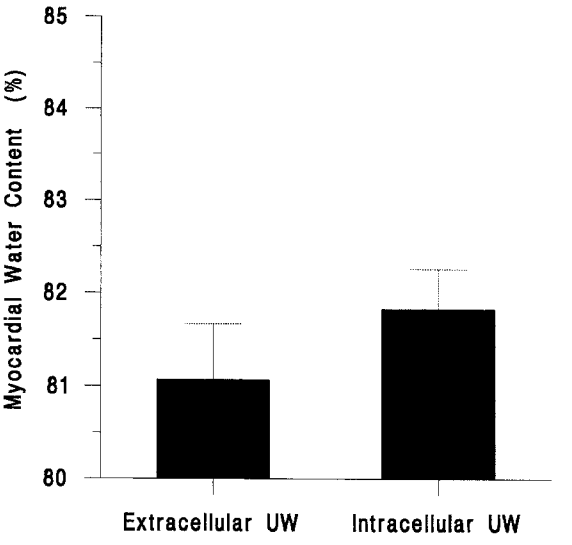

Fig. 4. No significant difference in myocardial water content was noted between extracellular UW and intracellular UW solutions.

that of the control (high-potassium UW) hearts (SWI 16.4 ± 1.9 × 10E3 erg/gm).

High-energy phosphates. High-energy phosphate levels are shown in Fig. 3. ATP, ADP, AMP, and creatinine phosphate levels were similar in both groups.

Ultrastructure. Electron micrographs of biopsy specimens obtained after functional assessment demonstrated similar findings in both groups. All hearts showed mild myofibrillar crowding and mild interstitial edema. In addition, there was mild mitochondrial enlargement and minimal nuclear chromatin margination.

Additional examination of capillary surfaces did not reveal major endothelial structural abnormalities. Some increase in the number of pinocytotic vesicles was noted in endothelial cells of both groups, but this did not correlate strongly with the preservation solution used.

Myocardial water content. Mean water content was similar in the two groups (Fig. 4).

Discussion

The optimal cardioplegic solution for heart transplantation must provide excellent myocardial preservation for up to 6 to 8 hours after a single administration given to arrest the heart. The duration of safe ischemic times allows for the ability to procure organs throughout North America.

Until mid-1990 the method of heart preservation used at our institution consisted of arrest with the Stanford cardioplegic solution followed by storage in cold normal saline solution. The Stanford solution includes dextrose to provide substrate for the ischemic heart, mannitol as an impermeant to prevent hypothermia-induced cell swelling, magnesium for membrane stabilization, and 30 mEq/L of potassium to induce rapid cardiac arrest.[21] The Stanford solution has provided acceptable myocardial preservation for short to moderate duration (less than 6 hours) ischemic times.

The UW solution was developed by Wahlberg, Southard, and Belzer[22] for pancreas preservation. It has provided excellent laboratory[2, 3] and clinical[4, 23] results for kidney and liver transplantation. Several laboratory studies that evaluated UW for heart preservation have shown superior myocardial preservation in comparison with results with other cardioplegic solutions.[6-8] These results prompted randomized clinical trials,[10, 24] which led to the adoption of UW solution for heart preservation at several centers, including our own.

Because the presence of many of the components of UW solution was based on theory, several studies have been conducted to determine which components are essential to the solution.[7, 25, 26] These studies have concluded that the impermeants lactobionate and raffinose, the colloid hydroxyethyl starch (Pentafraction, DuPont Pharmaceuticals, Wilmington, Del.), adenosine as substrate for ATP production, and the free radical scavenger glutathione all contribute to the effectiveness of the UW solution.

Most studies that evaluated the UW solution have

also concluded that its intracellular composition (the high potassium concentration) contributes significantly to the efficacy of preservation.[5, 9, 27] This argument is based largely on findings of early studies that used the high-potassium Collins or Sacks solutions,[28] which resulted in improved functional recovery in comparison with the recovery when simple extracellular solutions were used.

Previous investigators have evaluated the efficacy of an extracellular UW solution. Moen and associates[29] demonstrated equal or slightly more effective preservation of the canine liver, kidney, and pancreas with an extracellular UW solution. Oka and colleagues[30] have shown good lung preservation with a low-potassium UW formulation.

Few groups have evaluated a low-potassium UW solution for heart preservation. Howden and associates[31] evaluated an extracellular UW solution (potassium 35 mEq/L) without hydroxyethyl starch in a heterotopic rat heart transplant model and reported no difference in survival with the standard UW solution. Actual myocardial function could not be assessed in this model. Okuchi and associates[32] compared a low-potassium (30 mEq/L) solution with the glucose-insulin-potassium and Bretschneider solutions and concluded that the modified UW solution was the most effective but made no comparison with conventional UW solution. Ko and colleagues[27] found no decline in most myocardial parameters with the exception of elastance in canine hearts stored for 6 hours with potassium and sodium reduced UW solution as compared with controls. We believe our study is the first to make a direct functional comparison between extracellular and intracellular UW solutions for heart preservation.

Our results indicate equivalent functional recovery with extracellular or intracellular UW solution. The only difference between the two solutions in our study was the concentrations of potassium and sodium. Support for the belief that the intracellular nature of UW solution contributes significantly to its protective effect was not demonstrated in this experiment. It is argued that an intracellular storage solution will prevent hypothermia-induced cell swelling by preventing flux of sodium and potassium across the cell membrane. Anaerobic hypothermia suppresses the activity of the sodium-potassium pump, allowing sodium to enter the cell and inducing the swelling of the cell by accumulation of water. We hypothesize that the osmotic force of the impermeants lactobionate, raffinose, and pentastarch found in UW prevents the cell swelling seen with simpler extracellular preservation solutions and eliminates the need for intracellular composition.

This study was initially prompted by our observation of increased cardiac allograft vasculopathy in patients whose hearts were preserved with UW solution.[20] We have not established a causal link between hyperkalemic cardioplegic solutions and the development of cardiac allograft vasculopathy, and our study was retrospective and nonrandomized. However, the link between hyperkalemic cardioplegic solutions, including UW in particular, and endothelial dysfunction has been established. Olinger and colleagues[33] studied the effect of exposure of monkey cephalic vein grafts to hyperkalemic solution on subsequent cholesterol accumulation. That group observed a significant increase in cholesterol deposition 12 weeks after operation in vein grafts filled with hyperkalemic cardioplegic solution (potassium 27 mEq/L) for 30 minutes compared with that in control grafts filled with normokalemic cardioplegic solution. Many recent studies have shown structural[11, 34] and functional[12-15] damage after infusion of hyperkalemic cardioplegic solutions. Hoover and colleagues[11] perfused canine jugular vein grafts with hyperkalemic cardioplegic (potassium 40 mEq/L) in one half of the vein and with lactated Ringer's solution in the other half and grafted the vein to the carotid artery. Light microscopy revealed intense adventitial fibrosis and appearance of fibroblast-like cells in the outer layer of the vein wall in the segment infused with hyperkalemic cardioplegic solution. Mankad, Chester, and Yacoub[13] found that raising the concentration of potassium in the cardioplegic solution from 20 to 30 mEq/L produced a significant abolition of endothelial response to 5-hydroxytryptamine and nitroglycerin-induced vasodilatation. Cartier and colleagues[14] showed inhibition of endothelium-dependent coronary artery relaxation in response to 5-hydroxytryptamine and nitroprusside infusion after a single dose of cold UW. Pearl and associates[15] evaluated multidose UW as an alternative to blood cardioplegia and found a similar complete loss of response to bradykinin and nitroprusside infusion and a loss of endothelial nitric oxide release. We are now repeating that study with our extracellular UW formulation.

Endothelial damage has been implicated in the development of atherosclerosis. Cartier and associates[35] found that regenerating denuded endothelium has abnormal function up to 8 weeks after

injury, which predisposes the vessel to vasospasm and thrombosis.

The concentration of potassium of 25 mEq/L was chosen as a compromise between the prevention of endothelial damage and the need to ensure rapid cardiac arrest. It was noted that the hearts that received low-potassium UW solution arrested rapidly but generally not as quickly as those that received the high-potassium solution. Rapid arrest is desirable to ensure the presence of large ATP stores during cold storage.

Future studies may evaluate solutions with potassium concentrations lower than 25 mEq/L, because some endothelial dysfunction has been observed with potassium levels close to 25 mEq/L.[13, 34] We recently demonstrated that multidose UW solution is superior to multidose blood cardioplegia for intraoperative myocardial protection.[36] Multidose extracellular UW solution may provide similar protection without inducing endothelial dysfunction. Low-potassium UW solution may also have benefits in liver transplantation, eliminating the need for flushout of the liver before reperfusion.[29] Extracellular UW solution provides equivalent myocardial protection and appears to decrease the likelihood of endothelial injury. Future studies will specifically compare endothelial function with the two solutions. A randomized clinical trial is presently planned.

REFERENCES

1. Opelz G, Wujciak T. The influence of HLA compatibility on graft survival after heart transplantation: the Collaborative Transplant Study. N Engl J Med 1994; 330:816-9.
2. Jamieson NV, Sundberg R, Lindell S, et al. Preservation of the canine liver for 24-48 hours using simple cold storage with UW solution. Transplantation 1988; 46:517-22.
3. Ploeg RJ, Goosens D, Vreugenhil P, McAnulty JF, Southard JH, Belzer FO. Successful 72-hour cold storage of dog kidneys preserved with UW solution. Transplant Proc 1988;48:191-6.
4. Kalayoglu M, Sollinger HW, Straata RJ, et al. Successfully extended preservation of the liver for clinical transplantation. Lancet 1988;1:617-9.
5. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation 1988; 45:673.
6. Swanson DK, Pasaoglu I, Berkhoff HA, Southard JH, Hegge JO. Improved heart preservation with UW solution. J Heart Transplant 1988;7:456-67.
7. Wicomb WN, Collins GM. Twenty-four hour rabbit heart storage with UW solution: effects of low-flow perfusion, colloid, and shelf storage. Transplantation 1989;48:6-9.
8. Stein DG, Permut LC, Drinkwater DC, Bhuta S. Complete functional recovery after 24-hour heart preservation with University of Wisconsin solution and modified reperfusion. Circulation 1991;84 (Suppl): III316-23.
9. Breda MA, Drinkwater DC, Laks H, et al. Successful long-term preservation of the neonatal heart with a modified intracellular solution. J THORAC CARDIOVASC SURG 1992;101:139-50.
10. Stein DG, Drinkwater DC, Laks H, et al. Cardiac preservation in patients undergoing transplantation: a clinical trial comparing University of Wisconsin solution and Stanford solution. J THORAC CARDIOVASC SURG 1991;102:657-65.
11. Hoover EL, Pett SB, Eldor A, et al. The effects of crystalloid potassium cardioplegic solution on arterialized canine vein grafts. Circulation 1981;64(Suppl): II96-100.
12. Cartier R, Pellerin M, Hollmann C, Pelletier LC. Effects of pressure and duration of hyperkalemic infusions on endothelial function. Ann Thorac Surg 1993;55:700-5.
13. Mankad PS, Chester AH, Yacoub MH. Role of potassium concentration in cardioplegic solutions in mediating endothelial damage. Ann Thorac Surg 1991;51:89-93.
14. Cartier R, Hollman C, Dagenais F, Buluran J, Pellerin M, Leclerc Y. Effects of University of Wisconsin solution on endothelium-dependent coronary artery relaxation in the rat. Ann Thorac Surg 1993;55:50-6.
15. Pearl JM, Laks H, Drinkwater DC, et al. Loss of endothelium-dependent vasodilation and nitric oxide release after myocardial protection with University of Wisconsin solution. J THORAC CARDIOVASC SURG 1994;107:257-64.
16. Chu A, Chambers DE, Lin CC, Kuehl WD, Cobb FR. Nitric oxide modulates epicardial coronary basal vasomotor tone in awake dogs. Am Physiol Soc 1992; 262:H17-23.
17. Azuma H, Ishikawa M, Sekizaki S. Endothelium-dependent inhibition of platelet aggression. Br J Pharmacol 1986;88:411-5.
18. Shimokawa H, Aarhus LL, Vanhoutte PM. Impaired endothelium-dependent relaxation to aggregating platelets and related vasoactive substances in porcine coronary arteries in hypercholesterolemia and atherosclerosis. Circ Res 1989;64:900-14.
19. Fish DR, Nabel EG, Selwyn AP. Responses of coronary arteries with acetylcholine. J Clin Invest 1988;81: 21-6.
20. Drinkwater DC, Rudis E, Laks H, et al. University of Wisconsin solution versus Stanford cardioplegic solution and the development of cardiac allograft vasculopathy. J Heart Lung Transplant. [In press].

21. Billingham ME, Baumgartner WA, Watson DC, et al. Distant heart procurement for human transplantation: ultrastructural studies. Circulation 1980;62 (Suppl): I11-9.
22. Wahlberg JA, Southard JH, Belzer FO. Development of a cold storage solution for pancreas preservation. Cryobiology 1986;23:477-82.
23. Jamieson NV, Johnston PS, O'Grady JG, et al. Clinical use of UW solution or a simplified liver preservation solution prior to transplantation in 179 human livers. Transplant Proc 1990;22:2189.
24. Jeevandam V, Barr ML, Auteri JS, et al. University of Wisconsin solution versus crystalloid cardioplegia for human donor heart preservation. J THORAC CARDIOVASC SURG 1992;103:194-9.
25. Southard JH, Van Gulik TM, Ametani MS, et al. Important components of the UW solution. Transplantation 1990;49:251-7.
26. Sumitomo R, Jamieson NV, Wake K, Kamada N. Twenty-four-hour rat liver preservation using UW solution and some simplified variants. Transplantation 1991;5:91.
27. Ko W, Zelano JA, Lazenby WD, Isom OW, Kneger KH. Compositional analysis of a modified University of Wisconsin solution for extended myocardial preservation. Circulation 1992;86(Suppl):II326-32.
28. Collins GM, Bravo-Sugarman M, Terasaki PI. Kidney preservation for transplantation: initial perfusion and 30 hours ice storage. Lancet 1969;2:1219-22.
29. Moen J, Claesson K, Pienaar H, et al. Preservation of dog liver, kidney, and pancreas using the Belzer-UW solution with a high-sodium and low-potassium content. Transplantation 1989;47:940-5.
30. Oka T, Puskas JD, Mayer E, et al. Low-potassium UW solution for lung preservation. Transplantation 1991;52:984-8.
31. Howden BO, Jablonski P, Rosenfeldt F, Marshall VC. Effective organ preservation with modified HES-free UW solution with lowered potassium content. Transplant Proc 1992;24:2274-5.
32. Okouchi Y, Shimizu K, Yamaguchi A, Kamada N. Effectiveness of modified University of Wisconsin solution for heart preservation as assessed in heterotopic rat heart transplant model. J THORAC CARDIOVASC SURG 1990;99:1104-8.
33. Olinger GN, Boerboom LE, Bonchek LI, Hutchinson LD, Kissebah AH. Hyperkalemia in cardioplegic solutions causing increased cholesterol accumulation in vein grafts. J THORAC CARDIOVASC SURG 1983;85: 590-4.
34. Follette DM, Buckberg GD, Mulder DG, Fonkalsrud EW. Deleterious effects of crystalloid cardioplegic solutions on cultured endothelial cells. Surg Forum 1980;31:53-5.
35. Cartier R, Pearson PJ, Lin PJ, Schaff HV. Time course and extent of recovery of endothelium-dependent contractions and relaxations after direct arterial injury. J THORAC CARDIOVASC SURG 1991;102:371-7.
36. Pearl JM, Laks H, Drinkwater DC, et al. Normocalcemic blood or crystalloid cardioplegia provides better myocardial protection than does low-calcium cardioplegia. J THORAC CARDIOVASC SURG 1993;105: 201-6.